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DEHYDRODIFERULIC ACIDS FROM SUGAR-BEET PULP

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Key Word Index—*Beta vulgaris*; Chenopodiaceae; sugar-beet pulp; ferulic acid; diferulic acids; dehydrodiferulic acids.

Abstract—Sugar-beet pulp was found to contain dehydrodimers of ferulic acid, which were characterized and quantified by GC and GC-mass spectrometry. These compounds arise from 8–5′, 5–5′, 8–8′ and 8–O-4′ coupling, the 8–5′ form being preponderant; no 4–O-5′ dimer was detected. Total dehydrodimers represented 0.14% (w/w) of the pulp. Their release was attempted by treatment of the pulp with a commercial pectinase. However, diferulates were resistant to this enzymic hydrolysis; free diferulic acids were not released and remained esterified to residual structures of the sugar-beet pectins. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Pectins of sugar-beet carry ferulic acid groups, esterified mainly by the O-2 of arabinose residues and, to a lesser extent, by the O-6 of galactose residues of side-chains [1]. Sugar-beet pulp contains 0.8% (w/w) of ferulic acid [2–4]. Sugar-beet pectins can be cross-linked by dimerization of the ferulic acids under the action of chemicals (e.g. ammonium persulphate [5, 6]) or enzymatic oxidants (e.g. peroxidase/hydrogen peroxide [7]). These dimers (dehydrodimers) differ from the light-induced dimers called 'photodimers' [8]. The first dehydrodimer of ferulic acid obtained *in vitro* from wheat flour 'pentosans' was 5–5′ coupled and is usually termed diferulic acid (Fig. 1) [9, 10]. It has also been isolated from various cell walls of monocots and dicots (Chenopodiaceae), mainly in the *trans,trans* configuration [10–20], either alone or ester-linked to sugars [21]. Recently, other dehydrodimers have been identified in the cell walls of monocots (cocksfoot, switchgrass, suspension-cultured corn); they arose from 8–5′, 8–8′ and 8–O-4′ linkages (Fig. 1) [22]. The 8–5′ form was always predominant and the 4–O-5′ form, which can be chemically synthesized, was absent [22].

Dehydrodiferulic acids represent one mechanism for covalent cross-links between wall polymers, possibly controlling the mechanical strength [23] and texture [24] of the cell wall. Polysaccharidases, endo-transglycosylases and proteases, which are able to cleave the backbone of cross-linked polymers, and non-specific aryl esterases, which can cleave ester bonds, such as the one linking polysaccharides to the diferulic bridge, have been identified in cell walls [25].

Up to now, only 5–5′ dehydrodiferulic acid had been detected in sugar-beet pulp [17]. We have previously quantified dehydrodiferulic acids from sugar-beet pulp by reverse-phase HPLC [3]. The aim of the present study was to investigate the occurrence of diferulic acids in sugar-beet pulp and the possibility of releasing them by enzymic treatment.

RESULTS AND DISCUSSION

The dehydrodiferulic acids from an alkaline extract of sugar-beet pulp were analysed by GC. Products were confirmed by GC-mass spectrometry (Fig. 2; Table 1). Dimers resulting from 8–5′, 5–5′, 8–8′ and 8–O-4′ radical couplings were detected. The 8–5′ coupling mode represented about 48% (w/w) of all the dimers, with two distinct dehydrodimers following the saponification procedure [22], an acyclic (**2**, 21%) and a cyclic (or benzofuran, **1**, 79%) form. Dehydrodimers resulting from 8–O-4′, 8–8′ and 5–5′ (**4**, **5** and **6**, **3**, respectively) represented 31%, 12% and 9% (w/w) of the total dehydrodimers. As found in grasses [22], no 4–O-5′ dimer was detected and 8–5′ dimers were predominant. The 5–5′ dehydrodiferulic acid, identified by Hartley and Harris [17] is, therefore, not the only dimer from sugar-beet pulp. In view of its formation from ferulate via radical mechanisms, it is unlikely that the 5–5′ dimer would predominate in any plant system. Studies on monocots have shown

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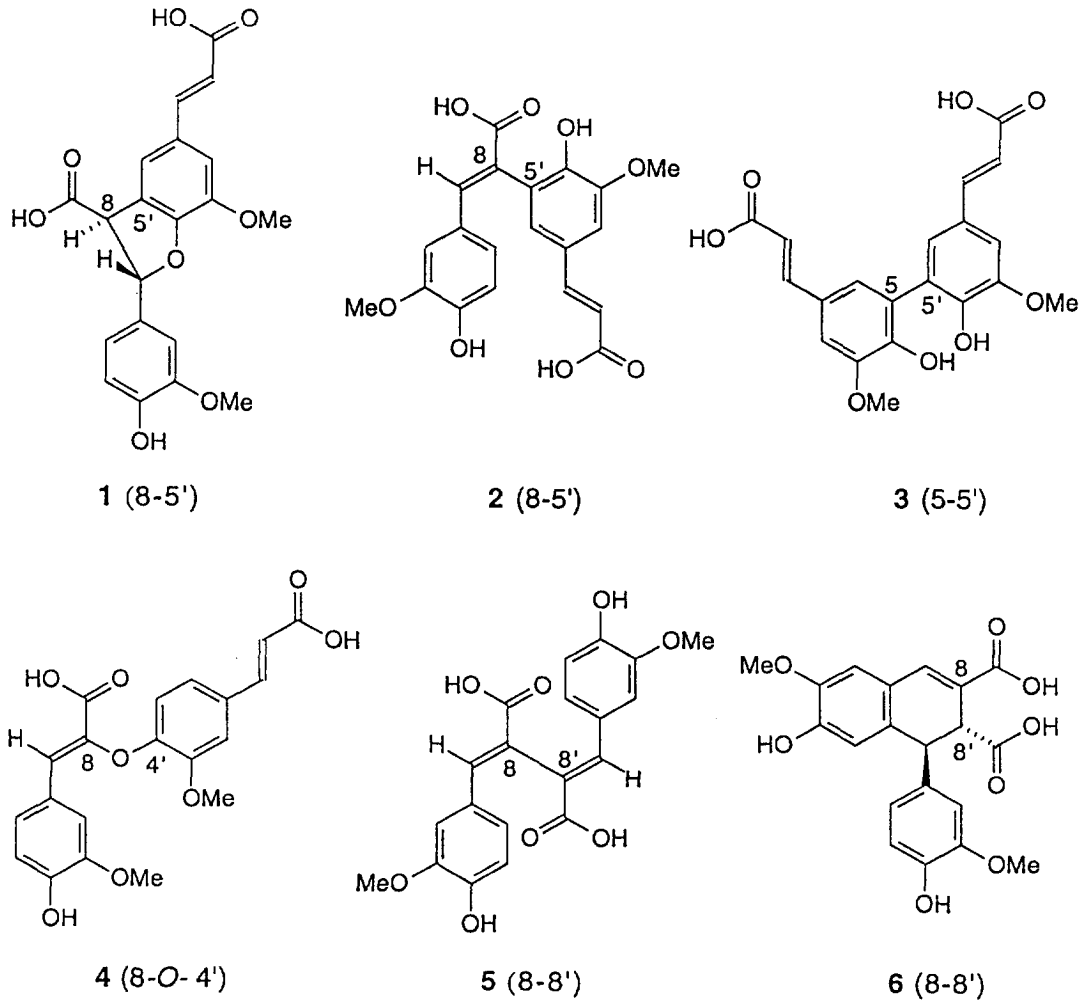

Fig. 1. Dehydrodiferulic acids: **1** and **2**, benzofuran and acyclic forms of 8–5′ coupling products; **3**, 5–5′ coupling or 'diferulic acid'; **4**, 8–O-4′ coupling; **5** and **6**, 8–8′ coupling.

that measuring only 5–5′ dimers led to up to a 20-fold underestimation of the total amount of dehydrodiferulic acids [22]. In sugar-beet pulp, we have shown that this factor is about 10.5.

The weight ratio of dehydrodiferulic acids to ferulic acid was 0.17, using response factors measured from authentic standards of each dimer. As the amount of ferulic acid in sugar-beet pulp is 0.8% [2], a dehydrodiferulic acid content of 0.14% can be calculated. We have previously [3] estimated the amounts of the dehydrodiferulic acids (0.04%) from sugar-beet pulp by HPLC (using a 5–5′ dimer as standard [26]), i.e. a diferulic acids/ferulic acid ratio of 0.05. This underestimation of dehydrodiferulic acids by HPLC was probably due to the assumption that all the dehydrodimers have the same extinction coefficient at 320 nm.

The sugar-beet pulp was hydrolysed by a pectinolytic preparation (SP 584) and the ether-extract of the soluble fraction of the enzymic hydrolysate analysed by GC-mass spectrometry. A strong ferulic acid peak was observed, but the peaks corresponding to the 8–5′, 5–5′, 8–8′ and 8–O-4′ dimers were absent.

However, when the soluble fraction of the enzymic hydrolysate of the sugar-beet pulp was saponified prior to GC analysis, all the dehydrodimers identified in the alkaline extract of sugar-beet pulp were then observed (Table 1). The 8–5′ compound was still present in acyclic (**2**) and cyclic (**1**) forms. The proportions of the various diferulate dimers in the saponification products of the soluble fraction of the enzymic hydrolysate were quite similar to those obtained in the alkaline extract of the beet pulp. 8–5′ dimers, and, to a lesser extent 8–O-4′ dimers, were the major ones, representing 38% and 32% (w/w) of the total dehydrodiferulic acids, respectively.

The fact that GC experiments showed striking differences when the soluble fraction of the enzymic hydrolysate was or was not treated with sodium hydroxide allowed us to conclude that: (i) dehydrodiferulic acids are present as esters in the enzymic hydrolysate; and (ii) since SP 584 hydrolysed the sugar-beet pectins [3], the dimers were initially linked to pectins such as ferulic acid. SP 584 possesses esterase activities that can release free ferulic acid from the pulp [3], but do not contain activities against the

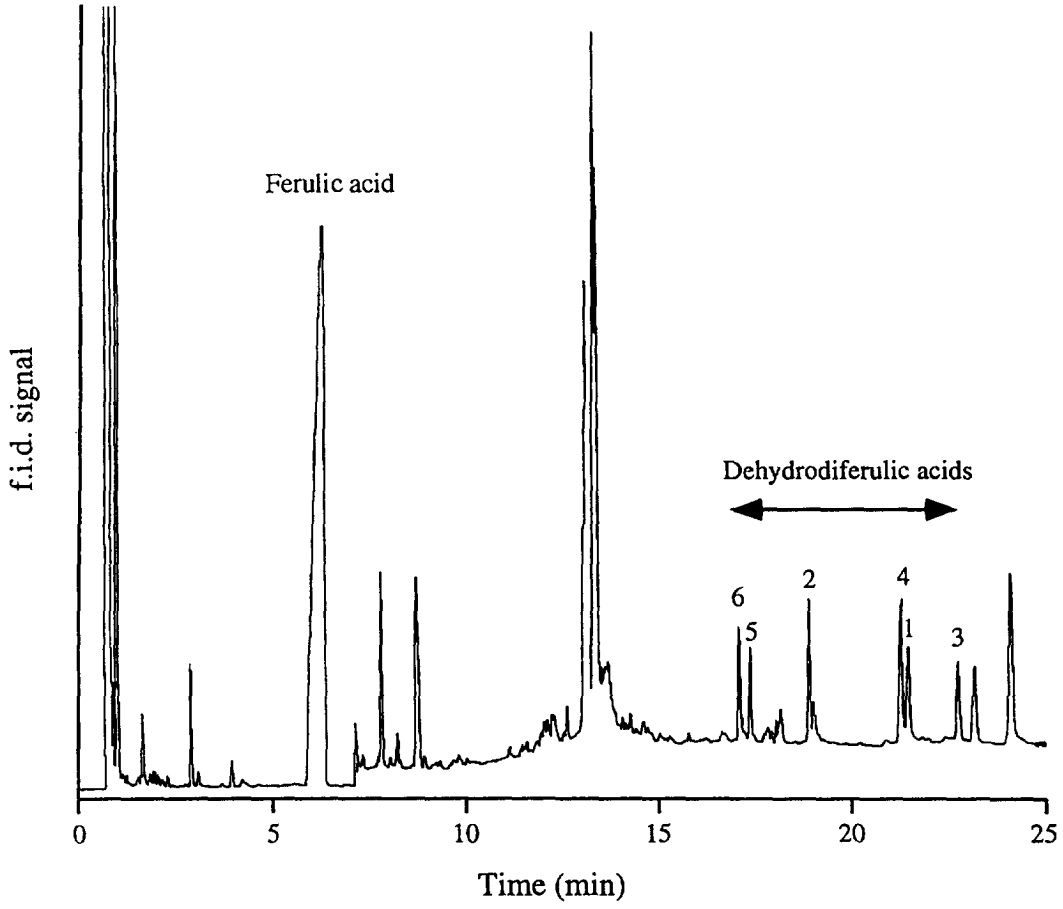

Fig. 2. GC of saponified sugar-beet pulp. Numbers refer to those in Fig. 1.

dimers. Therefore, the dehydrodiferulate cross-links appear to offer a structure that is resistant to hydrolysis by feruloyl esterases.

EXPERIMENTAL

Sugar-beet pulp. Dry matter 89.9%. From Sofalia (Chappes, France). An alcohol-insol. residue (AIR) was prepd as in [3].

Enzymic preparation. SP 584 was from Novo Nordisk A/S (Bagsvaerd, Denmark).

Standards. Ferulic acid was obtained from Fluka (Switzerland). 5–5′-Dehydrodiferulic acid (maize bran) was from the laboratory collection [26]. 8–5′ (**1** and **2**), 5–5′ (**3**) 8–O-4′ (**4**) and 8–8′ (**5** and **6**) dehydrodiferulic acids were obtained as described in ref. [22].

Hydrolysis. Hydrolysis of AIR by SP 584 was carried out according to ref. [3]. The soluble fr. of the enzymic hydrolysate was collected after filtration on a sintered glass filter (porosity 4). The pellets were used for other studies [27].

Saponification. Saponification of AIR (500 mg) and the soluble fr. of the enzymic hydrolysate of AIR (2 ml) was carried out under Ar and protected from light for 2 hr at 30° with 20 ml and 2 ml of 2 M NaOH,

Table 1. Amounts (% of total) of dehydrodiferulic acids in sugar-beet pulp

	Extraction method	
Diferulic acid (see Fig. 1)	Alkaline	Enzymic and alkaline
1 8–5′	38	26
	10	12
2 5–5′	9	12
3 8–O-4′	31	32
4 8–8′	4	4
	8	14

respectively. 2-Hydroxycinnamic acid (0.1 mg) was added as int. standard. The sample was acidified with 2 M HCl, extracted with 2 vols of Et_2O and dried under Ar.

Ether extraction. Ether extraction of the soluble fraction of the enzymic hydrolysate of AIR (2 ml, pH ~3) was done with 2 vols of Et_2O and drying under Ar.

GC and GC-MS analyses of dehydrodiferulic acids. Dried (Et_2O) extracts were treated as described in ref. [22].

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